REMARKS

Claims 1-7 were pending. Claims 1 and 7 have been amended; and claims 2-3 canceled. New claim 23 is added, support for which may be found in Example 1. No new matter is added. Applicants respectfully request reconsideration of the rejections.

Claims 1-7 have been rejected under 35 U.S.C. 112, second paragraph, as indefinite in the recitation of the term "control tissue". Applicants respectfully submit that the claims as written clearly define the invention.

As set forth in paragraph 62 of the specification, suitable control tissues include "normal cell populations, other regions of the diseased tissue, earlier or later time points in disease progression, *etc.*" The use of controls is well-known in the art, and a practitioner of ordinary skill can readily determine what samples, such as normal cell populations, spatially or temporally separated cells from the diseased tissue, and the like, are available and suitable for use as a control in any specific analysis.

Applicants respectfully submit that the present claims meet the requirements of 35 U.S.C. 112. Withdrawal of the rejection is requested.

Claims 1-7 have been rejected as anticipated by Schiffenbauer *et al.* (1997). The Office Action states that the methods of Schiffenbauer *et al.* comprise *in vivo* analysis of ovarian cancer tissues via MRI and analyzing images for specific features from tissues harvested from nude mice. Further, *in situ* hybridization is performed on frozen specimens for the detection of the expression of gene products such as mRNA.

Applicants respectfully submit that the presently claimed invention is not anticipated by the teachings of Schiffenbauer *et al.* The claims have been amended to clarify the nature of the subject invention. The present invention utilizes *in vivo* imaging, particularly dynamic contrast MRI, to detect spatial and temporal variations in the features of disease-associated tissues. The physical regions of the tissue that correlate with those imaging features are then assessed for patterns of gene and protein expression. The corresponding genes or gene products are useful in the design of therapeutic and/or diagnostic and imaging targets, with enhanced spatial and/or temporal specificity.

As set forth in the amended claims, a cellular sample from the individual is taken, and that same sample having observed imaging features in the dynamic contrast MRI is analyzed for gene expression. The present invention provides a means of determining how specific genes are changed within features observed with imaging, and therefore provide a means of obtaining

information from variations, *e.g.* variations in integrin expression, that correlate with what is observed with imaging. These methods permit a close relationship between the imaging methods and methods targeted to specific polypeptides. In contrast, the prior art teaches imaging, and then a non-specific analysis of gene expression from a different source of tissue.

The examples demonstrate the utility of the present methods. In Example 1, dynamic spiral breast MR imaging of 3 women showed substantial heterogeneity of the imaging characteristics over a single tumor. Samples were obtained from these same individuals following tumor resection, and information was obtained regarding the expression of target genes of interest.

In Example 2, *in vivo* dynamic contrast MR studies were performed on rabbits with palpable tumors. The rabbits were euthanized immediately following the last MR imaging experiment and the tumor tissues were harvested for immunohistochemical studies. The MR findings of a V2 carcinoma carrying rabbit injected with LM609-labelled AbPVs showed no immediate, 30 minutes and 1 hour post-contrast injection enhancement of the tumor or tumor margin occurs as compared to the pre-contrast image, whereas at 24 hours post-contrast injection, enhancement of the tumor margin was clearly visible. These results also show that the zone of $\alpha_v\beta_3$ upregulation seen on immunohistochemistry corresponds very well to the zone of enhancement seen on LM609-labelled AbPVs enhanced MRI. The stain is localized to the vessels and not distributed throughout the tumor cells

In Example 4, contrast-enhanced magnetic resonance imaging (MRI) was used to non-invasively characterize regions within the same tumor in order to provide a correlate for genomic analysis. The gene expression profiles of samples from a mouse tumor model obtained from contrast-enhancing and non-enhancing regions within the same tumor were compared using MRI and functional genomics. 11,000 genes from these samples were analyzed, and it was found that ten genes are upregulated in the contrast-enhancing areas, and one gene is upregulated in the non-enhancing region. Several of these genes encode extracellular matrix proteins. This study demonstrates that MRI can serve as a powerful, non-invasive tool for characterizing different regions of tumors to guide genomic analysis with both highspatial and temporal resolution.

The cited art does not anticipate the presently claimed invention. Schiffenbauer *et al.* examined with non-dynamic contrast MRI (using gradient echo MR images) the neovascularization of a tumor that was implanted in a host animal. Although imaging features were observed, these features were not then used to obtain corresponding cellular samples for gene expression analysis.

As stated in the article, the relative contributions of hormonal and stress induction of VEGF were analyzed in spheroids derived from MLS cells by in situ hybridization. As stated in the materials and methods section of the reference, "ovarian cancer cells (MLS, OC238) were plated at

a density of 50000 cells/flask on a 25-cm^2 tissue culture flask for preparation of conditioned medium as well as for analysis of VEGF expression." The gene expression analysis of Schiffenbauer *et al.* was therefore performed on cultures of cells grown *in vitro*, which were not directly correlated with the tumors obtained from animals, (which tumors were imaged).

Unlike the methods of the present invention, the prior art taught only that similar cells grown under vastly different conditions, *in vivo vs.* after hormonal stimulation in an in vitro setting, could be analyzed both by imaging and for gene expression. What the prior art fails to show, as taught by Applicants, is that gene expression in a specific tumor varies, and that the variation can be captured by selecting the specific features observed with imaging, and obtaining gene expression from samples correlating with the features.

Applicants respectfully submit that the cited art does not teach or anticipate the presently claimed invention. Withdrawal of the rejection is requested.

CONCLUSION

Applicants submit that all of the claims are now in condition for allowance, which action is requested. If the Examiner finds that a Telephone Conference would expedite the prosecution of this application, she is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any other fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815, order number STAN-182.

Respectfully submitted,

Date: July 27, 2004

By: Pamela Sherwood
Pamela J. Sherwood, Ph.D.
Registration No. 36,677

BOZICEVIC, FIELD & FRANCIS LLP
200 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: (650) 327-3400
Facsimile: (650) 327-3231